IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

E. MATSUTANI et al
Serial No. 09/806,125
Filed on March 28, 2001
For Agent That Retards Transformation of Hormone-Dependent Cancer to Non-Hormone-Dependent Cancer

Examiner: Stephen L. Rawlings, Ph.D.
Group Art Unit: 1642

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of
Patents and Trademarks,
P.O. Box 1450
Arlington, VA 22313-1450

Sir:

I, Mr. Kenichiro Naito, a citizen of Japan, and residing at 11-17, Segawa 2-chome, Minoo-shi, Osaka, 562-0045 JAPAN sincerely declare:

That I was born on June 30, 1958 in Ukyo-ku, Kyoto, Japan and graduated from the medical school, Osaka University, Japan, in March 1982;

That I have been employed by Takeda Pharmaceutical Company Limited, Osaka, Japan since April 1984, and have been engaged in pharmacological research at the Biology Research Laboratories of the Pharmaceutical Research Division of Takeda since 1984;

That I am a member of the Japanese cancer association, the molecular biology society of Japan and the Japanese society of hematology.

That I am one of the co-inventors of United States Patent Application Serial No. 09/806,125 filed 3/28/2001.

That under my direction and/or control, the following experiments were conducted:

Experimental Procedure

Cells of the human prostatic cancer cell line LNCaP in passage culture were trypsinized and suspended in RPMI1640 medium (Gibco BRL) containing 10% fetal bovine serum (BioWhittaker). The cell density of this cell suspension was determined using a CDA-500(Sysmex)and adjusted to 2 x 10^4 cells/ml using the medium described above. This suspension was dispensed to a 24-well multi-well culture plate (Becton Dickinson) at 0.5 ml per well and cultured at 37℃ in the presence of 5% CO_2 overnight.

To each well,

(i) 0.5 ml of a medium containing a preset concentration of 1 x 10^{-5} M cyproterone acetate (Sigma; Cat. No. C-3412),

(ii) 0.5 ml of a medium containing a preset concentration of cyproterone acetate and 0.4 μ M AG17 (Terada, H. 1981. Biochem. Biophys. Acta 639, 225),

(iii) 0.5 ml of a medium containing a preset concentration of cyproterone acetate and 20 μ M AG1024 (Ohmichi, M., et al. 1993. Biochemistry 32, 4650),

(iv) 0.5 ml of a medium containing a preset concentration of cyproterone acetate and 20 μ M AG1296 (Kovalenko, M., et al. 1994. Cancer Res. 54, 6106),

(v) 0.5 ml of a medium containing a preset concentration of cyproterone acetate and 20 μ M SU4984 (Mohammadi, M., et al. 1997. Science 276, 955), were added, respectively.

After cultivation at 37℃ in the presence of 5% CO_2 for 4 days, the number of cells in each well was determined using a CDA-500 (Table 1).

Results

Table 1

	Condition	Activity of Inhibiting Cell Growth (%)
(i)	5 x 10^{-6} M cyproterone acetate	20
(ii)	5 x 10^{-6} M cyproterone acetate + 0.2 μ M AG17	40
(iii)	5 x 10^{-6} M cyproterone acetate + 10 μ M AG1024	61
(iv)	5 x 10^{-6} M cyproterone acetate + 10 μ M AG1296	42
(v)	5 x 10^{-6} M cyproterone acetate + 10 μ M SU498475	75

The results show that a combination of cyproterone acetate and a tyrosine kinase possesses an exellent activity of inhibiting a cell growth of a non-hormone dependent prostatic cancer cell more than cyproterone acetate.

I further declare that all statements made herein are of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Osaka, Japan this th day of November, 2005

Kenichi NAITO

APPENDIX A

CALBIOCHEM®

AG 17

Size

Cat. No. 658425

5 mg

Synonyms: Tyrphostin A9; NSC 242557; (3,5-di-*t*-Butyl)-4-hydroxy-benxylidene Malononitrile; α-Cyano-(3,5-di-*t*-Butyl-4-hydroxy)cinnamonitrile; RG 50872

Description: Selective inhibitor of the platelet-derived growth factor receptor tyrosine kinase (IC_{50} = 500 nM). Anti-proliferative agent. Uncoupler of oxidative phosphorylation. Induces apoptosis in vitro and cell growth arrest in NHL cell lines.

Form: Off-white solid

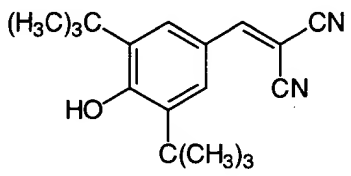
CAS Number: 10537-47-0

RTECS: OO3737000

Molecular Weight: 282.4

Molecular Formula: $C_{18}H_{22}N_2O$

Structure:



Purity: ≥99% by elemental analysis

Solubility: DMSO (10 mg/ml) and ethanol

Storage: Freezer (-20°C). Protect from light. Following reconstituion, aliquot and freeze (-20°C). This product is stable for 3 years as supplied. Stock solutions are stable for up to 1 month at -20°C.

Toxicity: MSDS available upon request.

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Revised: 01-May-98

コスモ・バイオ株式会社

U.S. Patent Application Serial No. 09/806,125

CN: 658425

References:

Burger, A.M., et al. 1995. *Cancer Res.* **55,** 2794.
Palumbo, G.A., et al. 1994. *Blood* **84,** 296a.
Bilder, G.E., et al. 1991. *Am. J. Physiol.* **260,** C721.
Levitzki, A., and Gilon, C. 1991. *Trends Pharmacol. Sci.* **12,** 171.
Terada, H. 1981. *Biochim. Biophys. Acta* **639,** 225

CALBIOCHEM®

SU4984

Size

Cat. No. 572625

1 mg

Synonym: 3[4-(1-Formylpiperazin-4-yl)-benzylidenyl]-2-indolinone

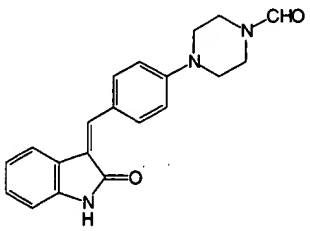
Description: Inhibits the tyrosine kinase activity of fibroblast growth factor receptor 1 (FGFR1; IC_{50} = 10 - 20 µM in the presence of 1 mM ATP) by interacting with the catalytic domain of FGFR1. Also inhibits aFGF-induced phosphorylation of ERK1 and ERK2 and tyrosine phosphorylation of PDGF and insulin receptors. Does not inhibit the activity of EGF receptor kinase.

Form: Orange-red solid

Molecular Weight: 333.4

Molecular Formula: $C_{20}H_{19}N_3O_2$

Structure:



Purity: ≥95% by HPLC

Solubility: DMSO (25 mg/ml)

Storage: Refrigerate (+4°C). Protect from light. Following reconstitution, aliquot and freeze (-20°C). This product is stable for 2 years as supplied. Stock solutions are stable for 3-4 weeks at -20°C.

Reference: Mohammadi, M., et al. 1997. *Science* **276,** 955.

Not available for sale in the United States.

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Revised: 20-February-03

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U.S. Patent Application Serial No. 09/806,125

CALBIOCHEM®

AG 1296

Size

Cat. No. 658551

5 mg

Synonyms: Tyrphostin AG 1296; 6,7-Dimethoxy-3-phenylquinoxaline

Description: Similar to but more potent than Tyrphostin AG 1295 (Cat. No. 658550). Inhibits signaling of human PDGF α-receptors (IC_{50} = 1.0 μM), β-receptors (IC_{50} = 800 nM), and the related stem cell factor receptor c-*kit* (80% inhibition at 5 μM). Has no effect on autophosphorylation of the vascular endothelial growth factor receptor KDR or on DNA synthesis induced by vascular endothelial growth factor in porcine aortic endothelial cells. Also reverses the transformed phenotype of *sis*-transfected NIH 3T3 cells but has no effect on *src*-transformed NIH 3T3 cells or on the activity of the kinase $p60^{c\text{-}src}$ (F257), which was immunoprecipitated from these cells. Reported to block the tyrosine kinase activity of FGF receptor.

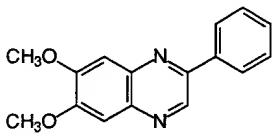
Form: Yellow solid

CAS Number: 146535-11-7

Molecular Weight: 266.3

Molecular Formula: $C_{16}H_{14}N_2O_2$

Structure:



Purity: ≥98% by HPLC

Solubility: DMSO (10 mg/ml)

Storage: Freezer (-20°C). Protect from light. Following reconstitution, aliquot and freeze (-20°C). This product is stable for 3 years as supplied. Stock solutions are stable for up to 1 month at -20°C.

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Revised: 31-October-02

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U.S. Patent Application Serial No. 09/806,125

CN: 658551

References: Strutz, F., et al. 2001. *Kidney Int.* **59,** 579.
Kovalenko, M., et al. 1994. *Cancer Res.* **54,** 6106.

CALBIOCHEM®

AG 1024

Size

Cat. No. 121767

1 mg

Synonym: Tyrphostin AG 1024

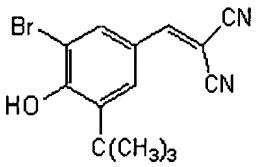
Description: A specific inhibitor of insulin-like growth factor-1 (IGF-1) and insulin receptor kinases with significantly lower IC_{50} values for IGF-1 than for insulin receptors. Induces apoptosis by downregulating the expression of phospho-Akt1 and Bcl-2; and increasing the expression of Bax, p53 and p21. Reported to enhance the radiosensitivity of human breast cancer cells.

Form: Off-white to yellow solid. Packaged under an inert gas.

Molecular Weight: 305.2

Molecular Formula: $C_{14}H_{13}BrN_2O$

Structure:



Purity: ≥98% by HPLC

Solubility: DMSO (10 mg/ml)

Storage: Freezer (-20°C). Protect from light. Following reconstitution, aliquot and freeze at -20°C. This product is stable for 2 years as supplied. Stock solutions are stable for 2 months at -20°C.

References:

Wen, B., et al. 2001. *Br. J. Cancer* **85,** 2017.
Parrizas, M., et al. 1997. *Endocrinology* **138,** 1427.
Ohmichi, M., et al. 1993. *Biochemistry* **32,** 4650.

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Revised: 04-February-02

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